

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

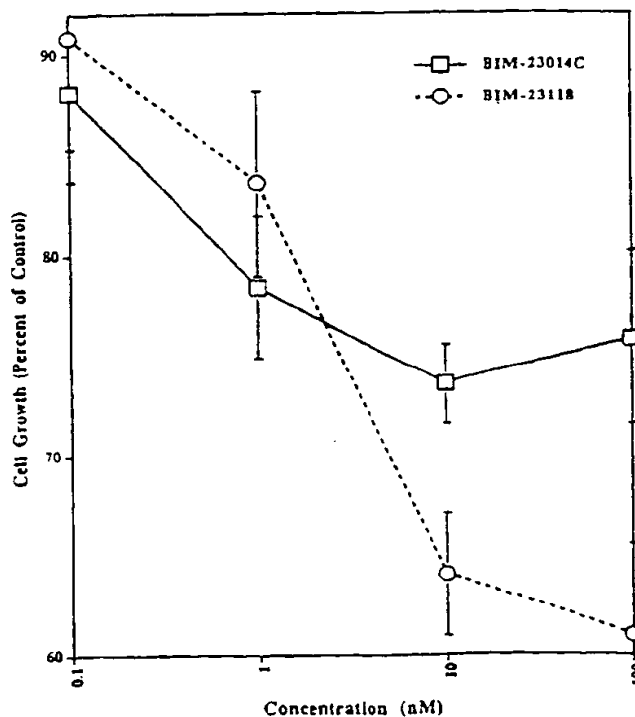
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 5 :</b> C07K 5/04, 7/10, 7/34, 7/36, 7/44, 7/26, 7/38, 7/12, A61K 37/24, 37/28, 37/40		<b>A1</b>	<b>(11) International Publication Number:</b> WO 95/04752 <b>(43) International Publication Date:</b> 16 February 1995 (16.02.95)
<b>(21) International Application Number:</b> PCT/US94/08875 <b>(22) International Filing Date:</b> 8 August 1994 (08.08.94) <b>(30) Priority Data:</b> 08/104,194      9 August 1993 (09.08.93)      US <b>(71) Applicant:</b> BIOMEASURE, INC. [US/US]; 27 Maple Street, Milford, MA 01757 (US). <b>(72) Inventors:</b> KIM, Sun, Hyuk; 20 Whitney Street, Chestnut Hill, MA 02167 (US). DONG, Zhengxin; 40 Angelica Drive, Framingham, MA 01701 (US). TAYLOR, John, E.; 74 Fiske Mill Road, Upton, MA 01568 (US). MOREAU, Sylviane; 159 Westboro Road, Upton, MA 01568 (US). KEYES, Susan, Riley; Apartment 3, 41 Pinckney Street, Boston, MA 02114 (US). <b>(74) Agent:</b> CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).			<b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** THERAPEUTIC PEPTIDE DERIVATIVES**(57) Abstract**

Peptide derivatives containing one or more substituents separately linked by an amide, amino or sulfonamide bond to an amino group on either the N-terminal end or side chain of a biologically active peptide moiety. The peptide derivatives have relatively enhanced biological activity when compared to the corresponding peptide alone.

**Effect of Somatostatin Analogs on the Proliferation of AR42J Rat Pancreatic Tumor Cells**



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## THERAPEUTIC PEPTIDE DERIVATIVES

### Background of the Invention

This invention relates to therapeutic peptides.

5        Several attempts have been made to prolong the activity of biologically active peptides. For example, peptides have been chemically modified by synthetically adding sugar moieties to increase the period during which the peptide is active (Sandoz, WO 88/02756; Sandoz, WO  
10 89/09786; DE 3910667 A1, EPO 0 374 089 A2 (1990); and Breipohl, U.S. Patent No. 4,861,755 (1989)). The addition of cationic anchors (EPO 0 363 589 A2 (1990)) and lipid moieties (Whittaker, WO 91/09837; Jung, U.S. Patent No. 4,837,303 (1989)) has also been used to  
15 increase the lifetime of the peptide.

### Summary of the Invention

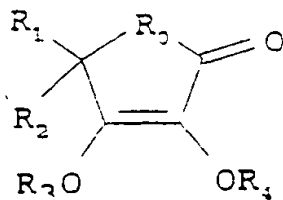
In general, the present invention provides derivatives of biologically active peptides which contain one or more substituents separately bonded to an amino  
20 group located on the N-terminal end or a side chain of the peptide moiety. In this modified form, the derivatives have more potent and prolonged biological activity than the corresponding unmodified peptide.

The peptide derivatives are advantageous in that  
25 they are inexpensive, highly biocompatible, lack deleterious side effects, and are compatible with different forms of therapeutic administration. In particular, many of the derivatives which have somatostatin as the peptide moiety have improved greatly  
30 improved potency and selectivity compared to unmodified somatostatin.

In one aspect, the invention features a peptide derivative containing a biologically active peptide moiety and at least one substituent attached to the

- 2 -

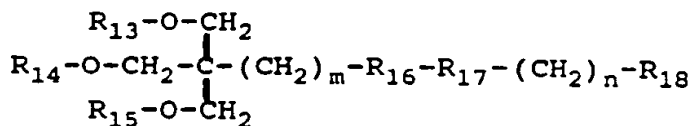
peptide moiety; the substituent is selected from the group including Compounds I, II, and III, where Compound I is:



where:

- 5  $R_0$  is O, S, or  $NR_5$ , where  $R_5$  is H or  $(C_1-C_6)$  alkyl; each  $R_1$  and  $R_2$ , independently, is H,  $(CH_2)_mOR_6$ , or  $CH(OR_7)CH_2OR_8$ , where  $R_6$  is H or  $(C_2-C_7)$  acyl, and each  $R_7$  and  $R_8$ , independently, is H,  $(C_2-C_7)$  acyl, or  $C(R_9)(R_{10})$ , where each  $R_9$  and
- 10  $R_{10}$ , independently, is H or  $(C_1-C_6)$  alkyl; or each  $R_1$  and  $R_2$  is  $=CHCH_2OR_{11}$ , wherein in  $R_{11}$ , each  $R_1$  and  $R_2$ , independently, is H or  $(C_2-C_7)$  acyl, and m is an integer between 1 and 5, inclusive; and
- 15 one of  $R_3$  or  $R_4$  is  $(CH_2)_nR_{12}$  or  $(CH_2)_nCH(OH)R_{12}$ , where  $R_{12}$  is CO,  $CH_2$ , or  $SO_2$ , and n is an integer between 1 and 5, inclusive; and the remaining  $R_3$  or  $R_4$  is H,  $(C_1-C_6)$ hydroxyalkyl, or  $(C_2-C_7)$  acyl; and

20 Compound II is:



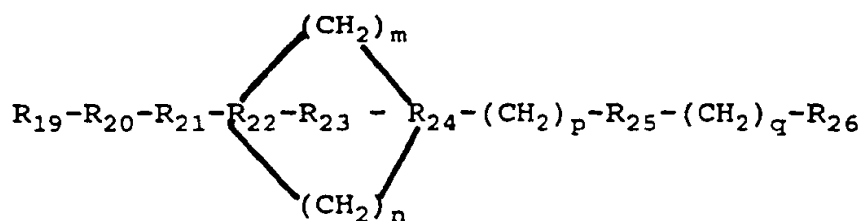
where:

- 25 each  $R_{13}$ ,  $R_{14}$  and  $R_{15}$ , independently, is H or  $(C_2-C_{24})$  acyl;
- $R_{16}$  is NH or absent;

- 3 -

$R_{17}$  is CO, O, or absent;  
 $R_{18}$  is CO,  $\text{CH}_2$ ,  $\text{SO}_2$ , or absent; and  
 $m$  is an integer between 1 and 5, inclusive; and  $n$   
 is an integer between 0 and 5, inclusive; and

5 Compound III is:



where:

- 10  $R_{19}$  is H,  $\text{NH}_2$ , an aromatic functional group, OH,  $(\text{C}_1\text{-C}_6)$  hydroxyalkyl,  $\text{H}(\text{R}_{27})(\text{R}_{28})$ ,  $\text{SO}_3\text{H}$ , or absent where each  $\text{R}_{27}$  and  $\text{R}_{28}$ , independently, is H or  $(\text{C}_1\text{-C}_6)$  alkyl;
- $\text{R}_{20}$  is O or absent;
- 15  $\text{R}_{21}$  is  $(\text{C}_1\text{-C}_6)$  alkyl or absent;
- $\text{R}_{22}$  is N, CH, O, or C;
- $-\text{R}_{23}-$  is  $(\text{C}_1\text{-C}_6)$  alkyl or absent;
- $\text{R}_{24}$  is N, CH, or C;
- $\text{R}_{25}$  is NH, O, or absent;
- 20  $\text{R}_{26}$  is  $\text{SO}_2$ , CO,  $\text{CH}_2$ , or absent;
- $m$  is an integer between 0 and 5, inclusive;
- $n$  is an integer between 0 and 5, inclusive;
- $p$  is an integer between 0 and 5, inclusive; and
- $q$  is an integer between 0 and 5, inclusive.

- 25 In Compounds I, II and III the peptide moiety is attached to each of the substituents by a CO-N,  $\text{CH}_2\text{-N}$ , or  $\text{SO}_2\text{-N}$  bond between the substituent and a nitrogen atom of the N-terminus or a side chain of said peptide moiety.

- 4 -

In preferred embodiments,  $-R_{23}-$  is  $(C_1-C_6)$ alkyl;  $R_{22}$  is N, C or CH; and  $R_{24}$  is C. Alternatively,  $R_{22}$  is O;  $R_{19}$ ,  $R_{20}$ ,  $R_{21}$ , and  $-R_{23}-$  are absent; and the sum of m and n is 3, 4, or 5.

- 5 In other preferred embodiments of the invention, the substituent is Compound I; in this embodiment,  $R_{12}$  is preferably  $CH_2$  or  $SO_2$ . Alternatively, the substituent may be Compound II, in which case  $R_{18}$  is preferably  $CH_2$  or  $SO_2$ ;  $R_{13}$ ,  $R_{14}$ , and  $R_{15}$  are H; and  $R_{17}$  is absent. In
- 10 particularly preferred embodiments, the substituent is  $(HOCH_2)_3C-NH-(CH)_2-SO_2$  or  $(HOCH_2)_3C-CH_2$ .

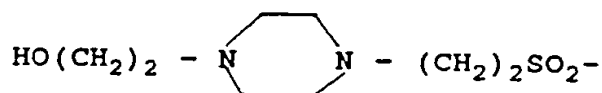
In still other embodiments of the invention, the substituent is Compound III; preferably, in this embodiment,  $-R_{23}-$  is absent and at least one of  $R_{22}$  and

15  $R_{24}$  is N. Alternatively, both  $R_{22}$  and  $R_{24}$  may be N.

In other embodiments, the substituent is one of:



and



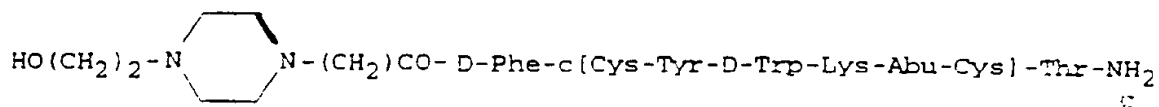
- 20 Preferably, the peptide moiety is selected from the group including: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte stimulating hormone (MSH),
- 25 adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GHRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, Bradykinin, glucagon-like peptide (GLP),
- 30 gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive

- 5 -

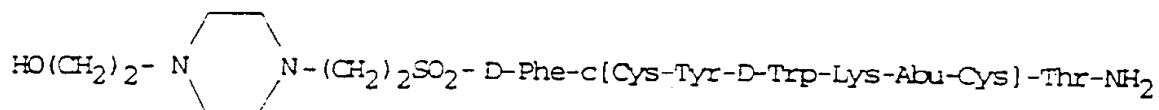
intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating polypeptide (PACAP), beta-cell tropin, adrenomedulin, and derivatives, fragments, and analogs thereof.

- 5 The peptide moiety is preferably somatostatin or a derivative, fragment, or analog thereof. Most preferably, the somatostatin analog is one of: H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH<sub>2</sub>, H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH<sub>2</sub>, and H-D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH<sub>2</sub>. Alternatively, the peptide moiety is bombesin or a derivative, fragment or analog thereof.

In still other preferred embodiments, the peptide derivative is one of:



15 and



- In another aspect, the invention provides a dimeric peptide derivative containing two biologically active peptide moieties, and at least one substituent attached to each of the peptide moieties. The substituent is selected from the group consisting of compounds IV and V, where compound IV has a generic structure equivalent to compound I, and compound V has a generic structure equivalent to compound III. In the dimer, each of the peptide moieties is attached to the substituents by a CO-N, CH<sub>2</sub>-N, or SO<sub>2</sub>-N bond between the substituent and a nitrogen atom of the N-terminus or a side chain of one of the peptide moieties.

In yet another aspect, the invention provides a method for treating a disease, such as cancer, in a

- 6 -

patient; the method includes the step of administering to the patient a therapeutic amount of the peptide derivatives described herein. In preferred embodiments, the peptide moiety used in the treatment is somatostatin.

5 By "biologically active", as used herein, is meant a naturally occurring, recombinant, and synthetic peptide having physiological or therapeutic activity. In general, this term covers all derivatives, fragments, and analogs of biologically active peptides which exhibit a  
10 qualitatively similar or opposite effect to that of the unmodified peptide.

#### Brief Description of the Drawings

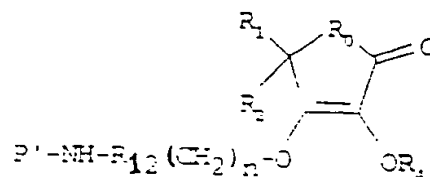
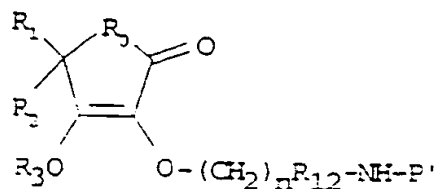
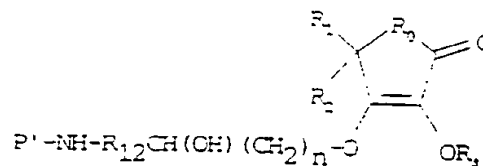
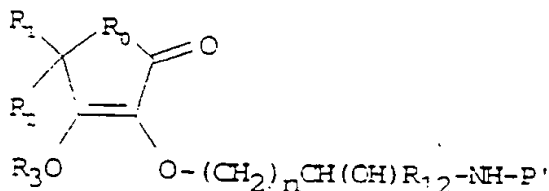
Fig. 1 is a graph of two growth curves of AR42J cells in the presence of different somatostatin  
15 derivatives.

#### Description of the Preferred Embodiments

##### Peptide Derivatives

In general, peptide derivatives of the invention contain two separate components: 1) a biologically active  
20 peptide; and, 2) at least one substituent having the structure of Compounds I, II, and III. Peptide derivatives made according to the methods described herein include the following compounds.

##### Compound I-Based Derivatives

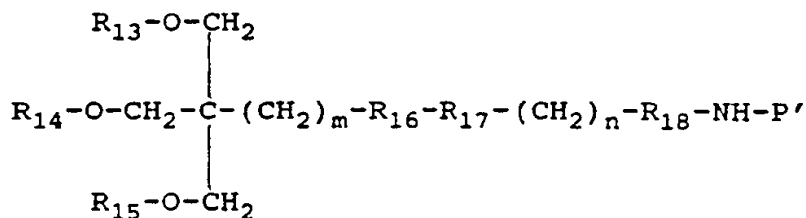




- 7 -

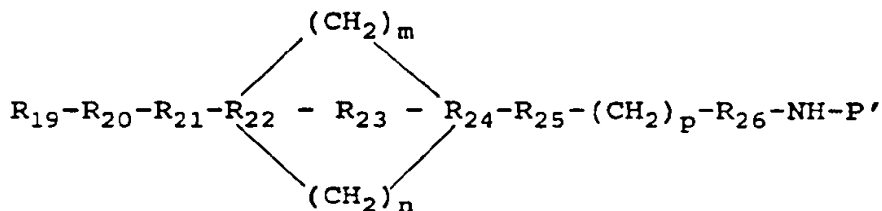
wherein  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_{12}$ , and  $n$  are as defined herein, and  $NH-P'$  is the biologically active peptide moiety. In these embodiments, the  $NH$  group is located on the N-terminal end or side chain of the peptide and  $P'$  represents the remainder of the peptide.

#### Compound II-based Derivatives



10 wherein  $R_{13}$ ,  $R_{14}$ ,  $R_{15}$ ,  $R_{16}$ ,  $R_{17}$ ,  $R_{18}$ ,  $m$ ,  $n$ , and  $NH-P'$  are as defined herein.

#### Compound III-Based Derivatives



15

wherein  $R_{19}$ ,  $R_{20}$ ,  $R_{21}$ ,  $R_{22}$ ,  $R_{23}$ ,  $R_{24}$ ,  $R_{25}$ ,  $R_{26}$ ,  $m$ ,  $n$ ,  $p$ , and  $NH-P'$  are as defined herein.

In addition to the structures shown above, compounds made according to the invention include peptide derivatives containing two or more substituents attached to one peptide moiety. These embodiments of the invention are derivatives of biologically active peptides which have more than one free amino group, e.g., a lysine residue.

25 The invention also provides dimeric peptide derivatives containing two peptide moieties bound to a

- 8 -

single substituent, e.g., two Bradykinin analogs bound to a substituent of Compound V.

The peptide derivatives of the invention are derivatives of biologically active peptides selected from the following group: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, bradykinin, glucagon-like peptide (GLP), gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating polypeptide (PACAP), beta-cell tropin, adrenomedulin, or derivatives, fragments, or analogs of any of the foregoing.

In especially preferred embodiments, the peptide moiety is somatostatin or a derivative, fragment, or analog of somatostatin. Somatostatin analogs which can be used in accordance with the present invention include, but are not limited to the following compounds:

H-D- $\beta$ -Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys- $\beta$ -Nal-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys- $\beta$ -Nal-NH<sub>2</sub>;  
H-D- $\beta$ -Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr;  
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr;  
H-Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr;  
H-Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr;  
H-Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr;

- 9 -

- H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol;  
 H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
 H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
 H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
 5 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH<sub>2</sub>;  
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
 Ac-D-Phe-Lys\*-Tyr-D-Trp-Lys-Val-Asp-Thr-NH<sub>2</sub>  
 Ac-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 10 Thr-NH<sub>2</sub>;  
 Ac-D-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-  
 NH<sub>2</sub>;  
 Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-  
 NH<sub>2</sub>;  
 15 Ac-D-hArg(Et)<sub>2</sub>-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
 Ac-L-hArg(Et)<sub>2</sub>-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-  
 NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 20 Thr-NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 Phe-NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 Thr-NH<sub>2</sub>;  
 25 Ac-L-hArg(CH<sub>2</sub>-CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 Thr-NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-  
 Cys-Thr-NH<sub>2</sub>;  
 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-  
 30 Cys-Thr-NH<sub>2</sub>;  
 Ac-hArg(CH<sub>3</sub>, hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-  
 Thr-NH<sub>2</sub>;  
 H-hArg(hexyl)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-  
 NH<sub>2</sub>;

- 10 -

- Ac-D-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;  
Ac-D-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH<sub>2</sub>;  
5 Propionyl-D-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH<sub>2</sub>;  
Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg(Et)<sub>2</sub>-NH<sub>2</sub>;  
Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
10 Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
Ac-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-D-hArg(CH<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH<sub>2</sub>;  
15 Ac-D-hArg(Et)<sub>2</sub>-D-hArg(Et)<sub>2</sub>-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH<sub>2</sub>;  
20 Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH<sub>2</sub>;  
Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH<sub>2</sub>;  
Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH<sub>2</sub>;  
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
25 H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH<sub>2</sub>;  
H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
30 H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH<sub>2</sub>;  
H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH<sub>2</sub>;  
H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH<sub>2</sub>;  
35 Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH<sub>2</sub>;

- 11 -

- H-D-Phe-Cys- $\beta$ -Nal-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH<sub>2</sub>;  
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);  
cyclo (Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);  
5 cyclo (Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);  
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);  
cyclo (Pro-Tyr-D-Trp-Lys-Thr-Phe);  
cyclo (Pro-Phe-D-Trp-Lys-Thr-Phe);  
cyclo (Pro-Phe-L-Trp-Lys-Thr-Phe);  
10 cyclo (Pro-Phe-D-Trp(F)-Lys-Thr-Phe);  
cyclo (Pro-Phe-Trp(F)-Lys-Thr-Phe);  
cyclo (Pro-Phe-D-Trp-Lys-Ser-Phe);  
cyclo (Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);  
cyclo (D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);  
15 cyclo (D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);  
cyclo (D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);  
cyclo (D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);  
cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);  
cyclo (Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);  
20 cyclo (Pro-Phe-D-Trp-4-Amphe-Thr-Phe);  
cyclo (N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);  
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);  
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);  
cyclo (Asn-Phe-D-Trp-Lys-Thr-Phe);  
25 cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-NH(CH<sub>2</sub>)<sub>4</sub>CO);  
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe- $\beta$ -Ala);  
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;  
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe);  
cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);  
30 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);  
cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);  
cyclo (Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);  
cyclo (Asn-Phe-Phe-D-Trp(NO<sub>2</sub>)-Lys-Thr-Phe-Gaba);  
cyclo (Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);  
35 cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);

- 12 -

- cyclo (Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);  
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-  
 Pro-Cys)-OH;  
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-  
 5 Pro-Cys)-OH;  
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-  
 Tpo-Cys)-OH;  
 cyclo (Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-  
 MeLeu-Cys)-OH;  
 10 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);  
 cyclo (Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);  
 cyclo (Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);  
 cyclo (Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH-  
 (CH<sub>2</sub>)<sub>3</sub>-CO);  
 15 cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);  
 cyclo (Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba); and  
 cyclo (Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba)

where Lys\* indicates an amide bridge formed between Lys\* and Asp.

- 20 The peptide compounds listed above are described in the following references, each of which is incorporated herein by reference:

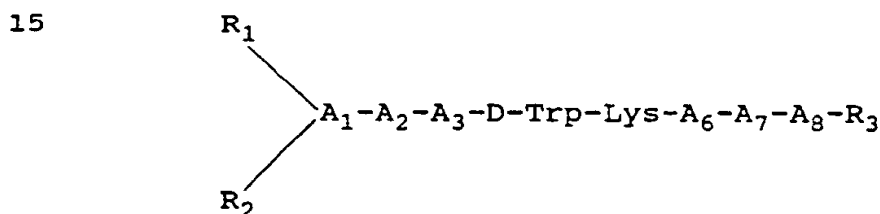
- EP Application No. P5 164 EU; Van Binst, G. et al. Peptide Research 5:8 (1992); Horvath, A. et al. Abstract,  
 25 "Conformations of Somatostatin Analogs Having Anti-tumor Activity", 22nd European Peptide Symposium, September 13-19, 1992, Interlaken, Switzerland; PCT Application WO 91/09056 (1991); EP Application 0 363 589 A2 (1990); EP Application 0 203 031 A2 (1986); U.S. Patent Nos.  
 30 4,904,642; 4,871,717; 4,853,371; 4,725,577; 4,684,620; 4,650,787; 4,603,120; 4,585,755; 4,522,813; 4,486,415; 4,485,101; 4,435,385; 4,395,403; 4,369,179; 4,360,516; 4,358,439; 4,328,214; 4,316,890; 4,310,518; 4,291,022;

- 13 -

4,238,481; 4,235,886; 4,224,190; 4,211,693; 4,190,648;  
4,146,612; and 4,133,782.

In the somatostatin analogs listed above, each amino acid residue has the structure of NH-C(R)H-CO-, in which R is the side chain; lines between amino acid residues represent peptide bonds which join the amino acids. When the amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated. When two Cys residues are present in the peptide, a disulfide bridge is formed between the two moieties. This bond, however, is not shown in the listed residues.

Additionally preferred somatostatin analogs of the invention are of the following formula:



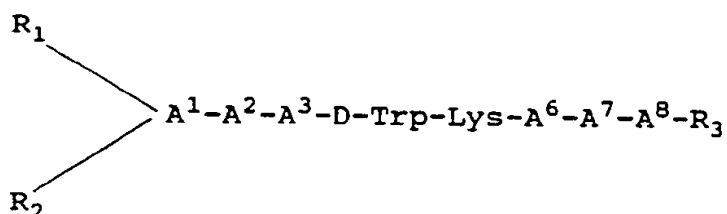
wherein  $A_1$  is a D- or L-isomer of  $\beta$ -Nal, Trp,  $\beta$ -pyridyl-Ala, Phe, substituted Phe, or deleted; and each  $A_2$  and  $A_7$ , independently, is Cys, Asp, or Lys. These moieties are covalently linked to each other via a disulfide bridge or an amide bridge. In addition,  $A_3$  is  $\beta$ -Nal, Phe, or o-, m-, or p-substituted X-Phe where X is a halogen, OH,  $NH_2$ ,  $NO_2$  or  $C_{1-3}$  alkyl;  $A_6$  is Val, Thr, Ser, Ala, Phe,  $\beta$ -Nal, Abu, Ile, Nle, or Nva; and  $A_8$  is Phe, Thr, Tyr, Trp, Ser,  $\beta$ -Nal, an alcohol group, or deleted; each  $R_1$  and  $R_2$ , independently, is H, lower acyl or lower alkyl; and  $R_3$  is OH,  $NH_2$ , or deleted. Preferably, when one of  $A_2$  and  $A_7$  is Cys, the other is also Cys; when  $A_8$  is an alpha-amino alcohol,  $R_3$  is deleted; and when neither of  $A_2$  and  $A_7$  is Cys,  $A_2$  is different from  $A_7$ .

- 14 -

Especially preferred somatostatin analogs of this embodiment are:

- Me-D-Phe-Cys-Tyr-Tyr-D-Trp-Lys-Val-Cys-Thr-NH<sub>2</sub>;  
 H-D-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH<sub>2</sub>;  
 5 H-D-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH<sub>2</sub>;  
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH<sub>2</sub>;  
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Nal-NH<sub>2</sub>; and  
 H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-ol.

In other embodiments, linear somatostatin analogs  
 10 of the invention have the following structure:



wherein A<sup>1</sup> is a D- or L- isomer of Ala, Leu, Ile,  
 15 Val, Nle, Thr, Ser, β-Nal, β-pyridyl-Ala, Trp, Phe, 2,4-  
 dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe,  
 wherein X is CH<sub>3</sub>, Cl, Br, F, OH, OCH<sub>3</sub> or NO<sub>2</sub>;

A<sup>2</sup> is Ala, Leu, Ile, Val, Nle, Phe, β-Nal,  
 pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-  
 20 Phe, or p-X-Phe, wherein X is CH<sub>3</sub>, Cl, Br, F, OH, OCH<sub>3</sub> or  
 NO<sub>2</sub>;

A<sup>3</sup> is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-  
 Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is  
 CH<sub>3</sub>, Cl, Br, F, OH, OCH<sub>3</sub> or NO<sub>2</sub>;

25 A<sup>6</sup> is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser;

A<sup>7</sup> is Ala, Leu, Ile, Val, Nle, Phe, β-Nal,  
 pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-  
 Phe, or p-X-Phe, wherein X is CH<sub>3</sub>, Cl, Br, F, OH, OCH<sub>3</sub> or  
 NO<sub>2</sub>;



- 15 -

A<sup>8</sup> is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, Phe,  $\beta$ -Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH<sub>3</sub>, Cl, Br, F, OH, OCH<sub>3</sub> or NO<sub>2</sub>, or an alcohol thereof; and  
5 each R<sub>1</sub> and R<sub>2</sub>, independently, is H, lower acyl or lower alkyl; and R<sub>3</sub> is OH, NH<sub>2</sub>, or deleted. Preferably, at least one of A<sup>1</sup> and A<sup>8</sup> and one of A<sup>2</sup> and A<sup>7</sup> must be an aromatic amino acid; and when A<sup>8</sup> is an alcohol, R<sub>3</sub> is deleted. Additionally, A<sup>1</sup>, A<sup>2</sup>, A<sup>7</sup> and A<sup>8</sup> cannot all be  
10 aromatic amino acids. Particularly preferred analogs of this aspect of the invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>;  
H-D-Phe-p-NO<sub>2</sub>-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH<sub>2</sub>;  
15 H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH<sub>2</sub>;  
H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH<sub>2</sub>;  
H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH<sub>2</sub>;  
H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH<sub>2</sub>; and  
20 H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-D- $\beta$ -Nal-NH<sub>2</sub>.

In still other preferred embodiments, the peptide moiety is bombesin or a derivative, fragment, or analog of bombesin. Bombesin analogs which can be used to  
25 practice the present invention include, but are not limited to, Neuromedin C, Neuromedin B, litorin, and gastrin-releasing peptide (GRP), which has the following amino acid sequence:

H-Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH<sub>2</sub>

Other bombesin analogs which may be used in the present invention include compounds described in the

- 16 -

following references, the contents of which are incorporated herein by reference:

- Coy et al. Peptides, Proceedings of the Eleventh Amer. Peptide Symposium, Ed. by Rivier et al. ESCOM, pp. 65-67 (1990); Wang et al. J. Biol. Chem. 265:15695 (1990); Mahmoud et al. Cancer Research 51:1798 (1991); Wang et al. Biochemistry 29:616 (1990); Heimbrook et al., "Synthetic Peptides: Approaches to Biological Problems", UCLA Symposium on Mol. and Cell. Biol. New Series, Vol. 86, ed. Tam and Kaiser; Martinez et al., J. Med. Chem. 28:1874 (1985); Gargosky et al., Biochem. J. 247:427 (1987); Dubreuil et al., Drug Design and Delivery, Vol 2:49, Harwood Academic Publishers, GB (1987); Heikkila et al., J. Biol. Chem. 262:16456 (1987); Caranikas et al., J. Med. Chem. 25:1313 (1982); Saeed et al., Peptides 10:597 (1989); Rosell et al., Trends in Pharmacological Sciences 3:211 (1982); Lundberg et al., Proc. Nat. Aca. Sci. 80:1120, (1983); Engberg et al., Nature 293:222 (1984); Mizrahi et al., Euro. J. Pharma. 82:101 (1982); Leander et al., Nature 294:467 (1981); Woll et al., Biochem. Biophys. Res. Comm. 155:359 (1988); Rivier et al., Biochem. 17:1766 (1978); Cuttitta et al., Cancer Surveys 4:707 (1985); Aumelas et al., Int. J. Peptide Res. 30:596 (1987); Szepeshazi. et al., Cancer Research 51:5980 (1991); Jensen, et al. Trends Pharmacol. Sci. 12:13 (1991); U.S. Patent Nos. 5,028,692; 4,943,561; 4,207,311; 5,068,222; 5,081,107; 5,084,555; EP Application Nos. 0 315 367 A2 (1989); 0 434 979 A1 (1991); 0 468 497 A2 (1992); 0 313 158 A2 (1989); 0 339 193 A1 (1989); PCT Applications Nos. WO 90/01037 (1990); 90/02545 (1992); and UK Application GB 1 231 051 A (1990).

The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically

- 17 -

acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl  
5 cellulose, and salts with inorganic acids such as hydrohalic acids, including hydrochloric acid, sulfuric acid, and phosphoric acid.

### Synthesis of Compounds

The syntheses of Compounds I, II and III are now  
10 described.

The following abbreviations are used in describing syntheses of compounds according to the present invention:

	Nal:	naphthylalanine (1 or 2)
15	Abu:	alpha-aminobutyric acid
	D:	dextrorotatory
	L:	levorotatory
	HOAC:	acetic acid
20	BOP:	benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluoro-phosphate
	BOC:	tert-butyloxycarbonyl
	DCC:	dicyclohexyl carbodiimide
	EDC:	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
25	DEPC:	diethylcyanophosphonate
	DMF:	dimethylformamide
	CH <sub>2</sub> CL <sub>2</sub> :	dichloromethane
	MeOH:	methanol
	EtOH:	ethanol
30	DIEA:	N,N-diisopropylethylamine
	HOBT:	1-hydroxybenzotriazole

- 18 -

HBTU: O-Benzotriazol-1-yl,N,N,N',N'-  
tetramethyluronium hexafluorophosphate

THF: Tetrahydrofuran

TFA: Trifluoroacetic Acid

5 Starting materials and intermediates for Compounds I, II, and III are commercially available. Alternatively, the starting materials can be easily prepared by methods which are well known and included in the literature. For example, the chemistry of ascorbic acid-related  
10 derivatives can be found in J. Chem. Soc., Perkin Trans. 1:1220 (1974); Carbohydr. Res., 67:127 (1978); Yakugaku Zasshi, 86:376 (1966); U.S. Pat. No. 4,552,888; J. Med. Chem., 31:793 (1988); *ibid.* 34:2152 (1991); and, 35:1618 (1992), the contents of which are incorporated herein by  
15 reference. The chemistry for tris-related derivatives can be found in Arch. Biochem. Biophys., 96, 653 (1962), Biochem., 5 467 (1966), the contents of which are also incorporated herein by reference.

#### Synthesis of Peptide Derivatives

20 In a general sense, the coupling of Compounds I, II, or III to an appropriate free amino group of a protected amino acid or peptide can be achieved according to well-known methods employed for peptide synthesis (e.g., DCC, DCC-HOBT, DIC-HOBT PPA, EDC-HOBT, DEPT, BOP,  
25 HBTU) using a base (e.g. DIEA) in an inert solvent (e.g. DMF, THF or CH<sub>2</sub>Cl<sub>2</sub> ethyl acetate or combination thereof). Deblocking of protected groups may also be carried out by well-known methods (e.g., removal of the group by the addition of acid or base, TFA, dioxan-HCl, ammonia,  
30 NaOMe, piperidine). In most cases, the reaction temperature should range from -30°C to room temperature.

In general, the first step of the synthesis involves the reaction between an epoxide and a free amino

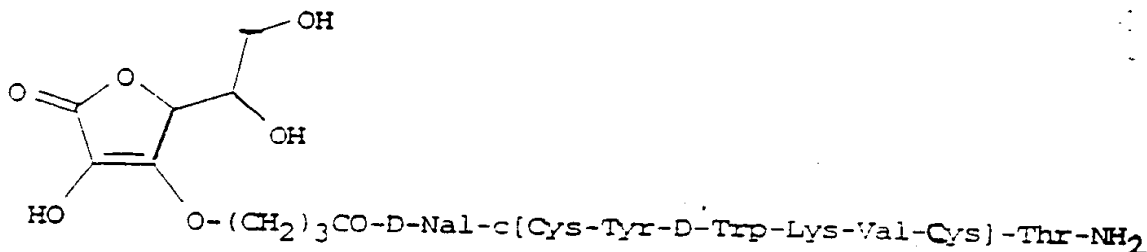
- 19 -

group of a protected amino acid or peptide; complexation and deprotection can be achieved utilizing well-known methods, such as those described in McManus, et al., Synth. Communications 3, 177 (1973), the contents of which are incorporated herein by reference. Following synthesis, purification of the intermediates and products can be achieved by conventional methods such as chromatography or HPLC. The identification of the compounds may be determined by conventional techniques such as NMR, amino acid analysis, and mass spectrometry.

The following Examples illustrate the preferred methods for forming the compounds of the invention.

#### Example 1 - Synthesis of Somatostatin Derivatives

The following somatostatin derivative, also referred to herein as BIM-23118, was synthesized in accordance with the invention:



#### Example 1.1 - 3-O-(Benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid

Acetic anhydride (6 ml) was added dropwise to a solution of 3-O-(benzyloxycarbonylmethyl)-ascorbic acid (2.2 g) in pyridine (30 ml); the mixture was then stirred overnight at room temperature. Pyridine was evaporated under reduced pressure leaving a residue which was then partitioned between ethyl acetate and 1N HCl. The ethyl acetate layer was washed with 1N HCl, and then water. After drying (MgSO<sub>4</sub>), the ethyl acetate was evaporated under reduced pressure; traces of pyridine and acetic anhydride which still remained were removed by multiple co-evaporations with toluene. The resulting 3-O-

- 20 -

(Benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid was dried under vacuum to yield a viscous gel which remained in the residue (2.4 g). TLC (silica gel:  $\text{CHCl}_3$ /acetone [9:1],  $R_f=0.52$ ).

5 Example 1.2 - 3-O-(carboxymethyl)-2,5,6-triacetyl-ascorbic acid

A slurry of Pd-C (100 mg) in water (2 ml) was added to a solution of 3-O-(benzyloxycarbonylmethyl)-2,5,6-triacetyl-ascorbic acid (2.4 g) in ethanol (30 ml),  
10 and the suspension was shaken under hydrogen (17 psi) for six hours. The catalyst was then removed by filtration through a celite pad and the filtrate evaporated under reduced pressure to yield 3-O-(carboxymethyl)-2,5,6-triacetyl-ascorbic acid. TLC (silica gel:  $\text{CHCl}_3$ /MeOH/HOAc  
15 [9:1:0.1],  $R_f=0.2$ ).

Example 1.3 - 5,6-O-Isopropylideneascorbic acid

Acetylchloride (0.67 ml) was added to a rapidly stirred suspension of ascorbic acid (8.0 g) in acetone (80 ml) and the mixture was stirred at room temperature  
20 overnight. The precipitate was collected by filtration, washed with ethyl acetate, and dried at reduced pressure to afford 8.29 g of 5,6-O-Isopropylideneascorbic acid as a colorless solid. TLC (silica gel:  $\text{CHCl}_3$ /MeOH/HOAc [3:1:0.1],  $R_f=0.54$ ).

25 Example 1.4 - 3-O-(Ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid

A solution of 5,6-isopropylidene ascorbic acid (2.0 g) in 10 ml DMF was added dropwise to a suspension of NaH (0.44 g of 50% mineral oil NaH dispersion washed  
30 with hexane several times) in 5 ml DMF. After gas evolution ceased, a solution of 1.43 ml ethyl 4-bromobutyrate in 5 ml DMF was added dropwise and the

- 21 -

mixture was stirred at room temperature overnight. Solvent was evaporated at reduced pressure and the resultant residue was chromatographed on silica gel (55 g) using  $\text{CHCl}_3/\text{MeOH}$  (19:1) as an eluant. Appropriate  
5 fractions were pooled and solvents removed at reduced pressure to yield a viscous residue containing 3-O-(Ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid (1.1 g).

Example 1.5 - 3-O-(carboxypropyl)-5,6-isopropylidene  
10 ascorbic acid

4.6 ml of 2N-NaOH was added to a solution of 3-O-(ethoxycarbonylpropyl)-5,6-isopropylidene-ascorbic acid (1.02 g) in 15 ml EtOH. After one hour, most of the ethanol was removed at reduced pressure and the residue  
15 was diluted with water (10 ml), and acidified with dil-HCL (pH 3). The solution was then saturated with NaCl and extracted several times with ethyl acetate; the pooled extracts were then dried using  $\text{MgSO}_4$ . Solvent was evaporated at reduced pressure to yield a viscous residue  
20 containing 3-O-(carboxypropyl)-5,6-isopropylidene ascorbic acid (0.84 g). TLC: (Silica gel:  $\text{CHCl}_3/\text{MeOH}/\text{HOAC}$  [5:1:0.1],  $R_f=0.55$ ).

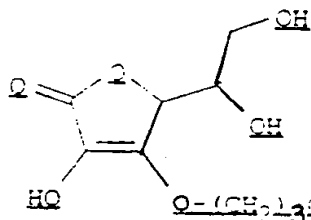
Example 1.6 - D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-  
Thr-NH<sub>2</sub>

25 A solution of di-tertbutyl dicarbonate (0.36 g) in 10 ml DMF was added dropwise to a solution of D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH<sub>2</sub> acetate (2 g, BIM-23014) in 45 ml DMF. After two hours at room temperature, solvent was removed under reduced pressure  
30 to yield a residue which was then chromatographed on silica gel (150 g) using  $\text{CHCl}_3/\text{MeOH}$ (9:1) as an eluant. Appropriate fractions were pooled and solvents removed under reduced pressure to yield a residue containing D-

- 22 -

Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH<sub>2</sub> (1.45 g).  
 TLC (silica gel: CHCl<sub>3</sub>/MeOH [3:1], R<sub>f</sub>=0.52).

Example 1.7 -



O-(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>-D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH<sub>2</sub>

- 0.2 ml diisopropylethylamine was added to a  
 5 solution of D-Nal-Cyclo-[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-  
 Thr-NH<sub>2</sub> (300 mg), 3-O-(carboxypropyl)-5,6-isopropylidene  
 ascorbic acid (56 mg) and HBTU (113 mg) in 5 ml DMF. The  
 mixture was then stirred at room temperature overnight,  
 and solvent was removed under reduced pressure. The  
 10 residue was partitioned between a mixture of ethyl  
 acetate/MeOH and a saturated aqueous NaCl solution, and  
 the ethyl acetate layer was washed with saturated aqueous  
 NaCl, then saturated aqueous NaHCO<sub>3</sub>, and then dried  
 (MgSO<sub>4</sub>). Solvent was evaporated under reduced pressure,  
 15 and the residue was subjected to preparative TLC using a  
 CHCl<sub>3</sub>/MeOH (8:1) mixture as a developing solvent. The  
 appropriate UV-positive zone was isolated and extracted  
 with CHCl<sub>3</sub>/MeOH. Solvents were removed at reduced  
 pressure to yield the above-identified product (0.20 g).  
 20 TLC (silica gel: CHCl<sub>3</sub>/MeOH[5:1], R<sub>f</sub>=0.54).

Example 1.8 - Removal of BOC Group

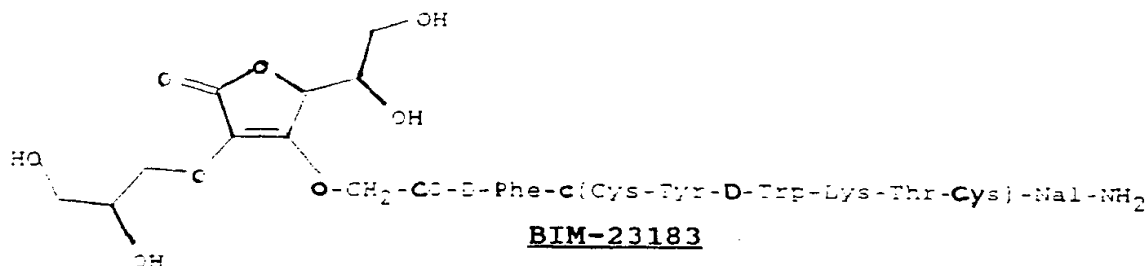
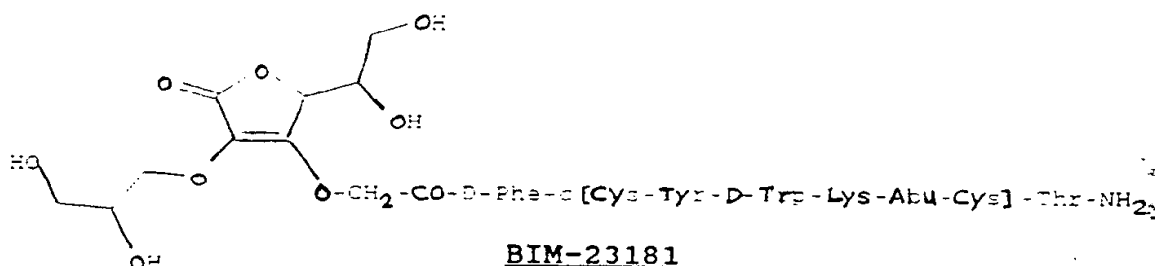
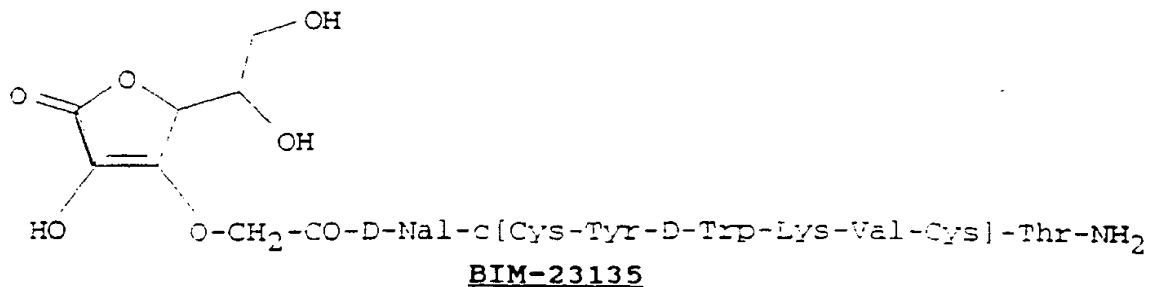
- The ascorbic acid derivative containing D-Nal-  
 c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH<sub>2</sub> (95 mg) shown  
 above was treated with 25% TFA in CHCl<sub>3</sub> for 45 min. at  
 25 room temperature. Volatile substances were removed under  
 reduced pressure to yield a dried residue which was  
 purified using Vydac C<sub>18</sub> HPLC and CH<sub>3</sub>CN/0.1% aqueous TFA.  
 The final yield was 90 mg (FAB-MS (m/e) 1341).



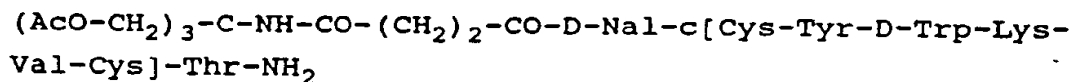
- 23 -

Example 1.9 - Other Embodiments

The following somatostatin derivatives were also synthesized in an analogous manner:

Example 2 - Synthesis of BIM-23107

The following somatostatin derivative, also referred to as BIM-23107, was synthesized in accordance to the invention.



Example 2.1 -  $(\text{AcO}-\text{CH}_2)_3-\text{C}-\text{NH}-\text{CO}-(\text{CH}_2)_2-\text{CO}-\text{D-Nal}-\text{c}[\text{Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys}]-\text{Thr}-\text{NH}_2$

0.03 ml DIEA was added to an ice-cooled solution of 2-N-(succinyl)amino-2-(acetoxymethyl)-1,3-propanediol

- 24 -

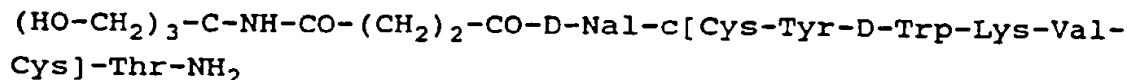
diacetate (83 mg) and HBTU (92 mg) in 2 ml of DMF. After stirring at 0-5° C for 30 minutes, a solution of D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH<sub>2</sub> (100 mg) in 2 ml DMF, containing 0.03 ml DIEA, was added. The mixture was first stirred at 0-5° C for one hour and then stirred at room temperature overnight. The solvent was removed at reduced pressure to yield a dried residue which was partitioned between ethyl acetate and aqueous saturated NaCl, and the EtOAc layer washed with aqueous 5% NaHCO<sub>3</sub>, and finally aqueous saturated NaCl; the resulting solution was then dried using MgSO<sub>4</sub>. The solvent was evaporated under reduced pressure leaving a residue containing (AcO-CH<sub>2</sub>)<sub>3</sub>-C-NH-CO-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Nal-c[Cys-Tyr-D-Trp-Lys(BOC)-Val-Cys]-Thr-NH<sub>2</sub> (0.14 gm). TLC (Silica Gel : CHCl<sub>3</sub>/MeOH/HOAc = 4:1:0.1, R<sub>f</sub>=0.82).

#### Example 2.2 - Removal of BOC group

30 mg of the above-identified compound was treated with 50% TFA in CHCl<sub>3</sub> for 45 minutes at room temperature; volatile substances were then removed at reduced pressure to yield a residue. Traces of TFA were co-evaporated with ethanol several times and the residue was titrated with ether and then dried to yield 30 mg of the product (30 mg). TLC (Silica gel: CHCl<sub>3</sub>/MeOH/HOAc = 3:1:1, R<sub>f</sub>=0.24).

#### Example 2.3 - Other Embodiments

The following somatostatin derivatives were also synthesized in an analogous manner.

BIM-23158

- 25 -

(HO-CH<sub>2</sub>)<sub>3</sub>-C-NH-CO-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH<sub>2</sub>

BIM-23167

(HO-CH<sub>2</sub>)<sub>3</sub>-C-NH-CO-(CH<sub>2</sub>)<sub>2</sub>-CO-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-  
5 Cys]-Thr-NH<sub>2</sub>

BIM-23173

(HO-CH<sub>2</sub>)<sub>3</sub>-C-NH-CH<sub>2</sub>-CO-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-  
Nal-NH<sub>2</sub>

BIM-23179

10 (HO-CH<sub>2</sub>)<sub>3</sub>-C-NH-CH<sub>2</sub>-CO-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-  
Thr-NH<sub>2</sub>

BIM-23182

Example 3 - Synthesis of BIM-23201

The following somatostatin derivative, also  
15 referred to as (BIM-23201), was synthesized in accordance  
with the present invention.

(HO-CH<sub>2</sub>)<sub>3</sub>-C-CH<sub>2</sub>-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH<sub>2</sub>

Example 3.1 - (HO-CH<sub>2</sub>)<sub>3</sub>-C-CH<sub>2</sub>-D-Phe-c[Cys-Tyr-D-Trp-Lys-  
Thr-Cys]-Nal-NH<sub>2</sub>-

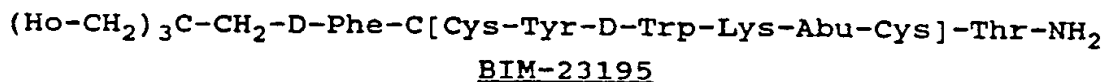
20 Two grams of 3Å molecular sieve followed by  
NaCNBH<sub>3</sub> (36 mg) were added portion-wise in 15 minute  
increments to a solution of D-Phe-c[Cys Tyr (OBt)-D-Trp-  
Lys(BOC)-Thr(OBt) Cys] Nal-NH<sub>2</sub> (250 mg) and tris  
(acetoxymethyl)acetaldehyde (120 mg) obtained by  
25 oxidation of triacetyl penta-erythritol with pyridinium  
dichromate or DMSO/oxalyl chloride/triethylamine) in  
methanol (10 ml) containing 10% acetic acid. The mixture

- 26 -

was then stirred at room temperature for 30 minutes and heated for 4 hours. After filtration, the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with water, then aqueous  $\text{NaHCO}_3$ ,  
 5 and then dried ( $\text{MgSO}_4$ ). The solvent was evaporated under reduced pressure to yield a residue (0.4 g) which was then dissolved in methanol (5 ml), treated with a  $\text{NaOMe/MeOH}$  solution (pH 10), stirred for 1 hour and finally neutralized with 1 N  $\text{HCl}$  to pH 5-6. After  
 10 evaporation of solvent, the residue was dissolved in 90% aqueous TFA (5 ml) and stirred for 30 minutes. Volatiles were removed at reduced pressure and traces of TFA and water in the resulting residue were removed by co-evaporation with alcohol (2x). The residue was dried,  
 15 then titrated with ether, and finally purified by HPLC using conditions similar to those described earlier, to yield 41 mg of  $(\text{HO-CH}_2)_3\text{-C-CH}_2\text{-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH}_2$  as a colorless solid. MS (m/e) 1262.8.

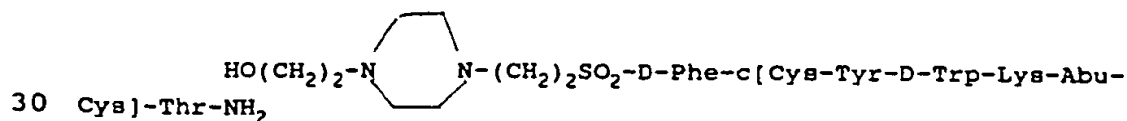
### Example 3.2 - Other Embodiments

20 The following somatostatin derivative, also referred to as BIM-23195, was synthesized in an analogous manner.



### 25 Example 4 - Synthesis of BIM-23197

The following somatostatin derivative, also referred to as BIM-23197, was synthesized in accordance with the invention.



- 27 -

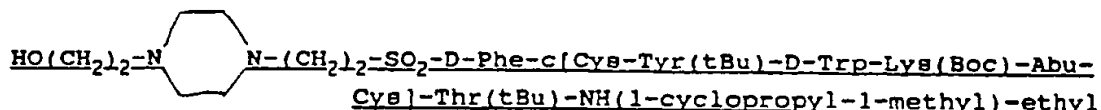
Example 4.1 - 2-Bromoethanesulfonyl Chloride

Na 2-Bromoethanesulfonate (4.0 g) was treated with  $\text{PCl}_5$  (11.8 g) while cooling in an ice bath. After reaching the liquid phase, the solution was heated at 90-  
5 120 °C for 1.5 hours in oil, cooled to room temperature, poured into 50 g of crushed ice, and then stirred for 15 min. The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 30 ml) and combined extracts were washed with  $\text{H}_2\text{O}$  (2 x), 5%  $\text{NaHCO}_3$  (2 x), and  $\text{H}_2\text{O}$  (2 x) again. Drying over anhydrous  
10  $\text{MgSO}_4$  and distillation under reduced pressure gave 2-bromoethanesulfonyl chloride as a colorless liquid (1.95 g, 42-44 °C/1 mm Hg).

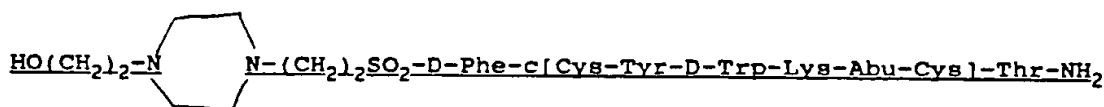
Example 4.2 - Br-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-methyl)-ethyl  
15

A solution of 2-bromoethane sulfonyl chloride (30 mg) in DMF (1 ml) was added dropwise to a solution of H-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-(1-cyclopropyl-1-methyl)-ethyl (150 mg) and DIEA (55 mg) in  
20 DMF (2 ml) under  $\text{N}_2$  at 0°C. The reaction mixture was stirred at 0-5 °C for 3 hours; solvent was then removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with 5% citric acid (2 x), 5%  $\text{NaHCO}_3$  (2 x) and brine (2 x). The solution was then  
25 dried over anhydrous  $\text{MgSO}_4$ , filtered, and condensed to dryness under reduced pressure. The product was further purified by a short silica gel column eluted with ethyl acetate. Fractions containing the product were pooled and the solvent was removed under reduced pressure,  
30 giving 105 mg of Br-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-methyl)-ethyl as a slightly yellow solid. (Silica gel,  $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$  (9:1:0.1),  $R_f=0.36$ ).

- 28 -

Example 4.3 -

A solution of Br-(CH<sub>2</sub>)<sub>2</sub>-SO<sub>2</sub>-D-Phe-c[Cys-Tyr(tBu)-D-Trp-Lys(Boc)-Abu-Cys]-Thr(tBu)-NH(1-cyclopropyl-1-methyl)-ethyl (100 mg) and 2-hydroxyethylpiperazine (55 mg) in 2 ml of 1-propanol was refluxed under N<sub>2</sub> for 2.5 hours. The solution was then cooled to room temperature, and the solvent was removed under reduced pressure. The residue was then dissolved in ethyl acetate containing 5% MeOH and washed with brine (3 x). Finally, the solution was dried over anhydrous MgSO<sub>4</sub>, filtered and condensed to dryness under reduced pressure, resulting in 110 mg of the above-identified solid. Without further purification, this compound was used directly in the next step.

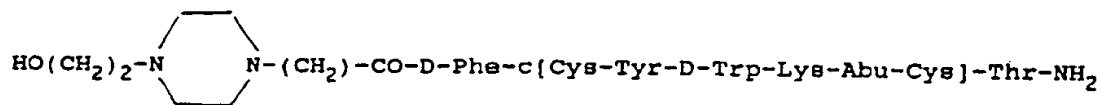
Example 4.4 -

110 mg of the protected somatostatin derivative obtained in the previous step was dissolved in 10 ml of 90% TFA aqueous solution, and stirred at room temperature under N<sub>2</sub> for one hour. TFA and H<sub>2</sub>O were removed under reduced pressure, and the residue was titrated with cold ether (3 x 10 ml). A slightly yellow solid was obtained; this material was further purified on preparative reverse phase HPLC, eluting with: 1) a NH<sub>4</sub>OAc aqueous solution; and, 2) an HOAc aqueous solution. Lyophilization of the pooled fractions containing the above-identified product gave a white solid. (18 mg. ESI-MS, ((m+1)/e) 1252.7).

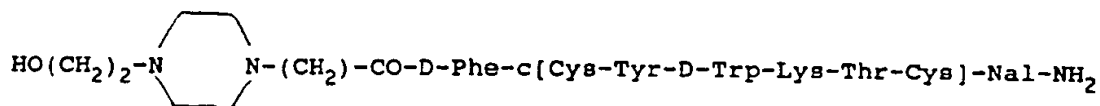
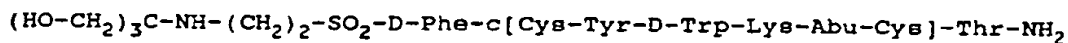
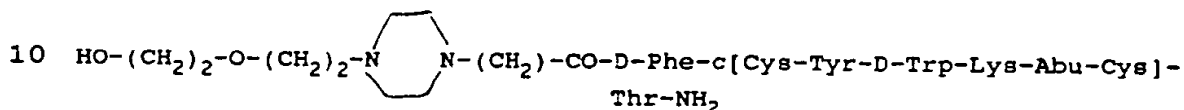
- 29 -

Example 4.5 - Other Embodiments

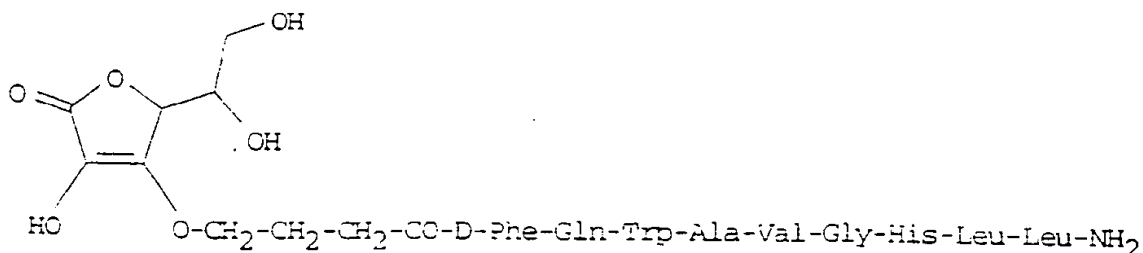
The following somatostatin derivatives were also synthesized in an analogous manner:



5

BIM-23190BIM-23191BIM-23196BIM-23202Example 5 - Synthesis of Bombesin Derivatives

The following bombesin derivative, also referred to as BIM-26333, was synthesized in an analogous manner as described above:



- 30 -

Other peptide derivatives of the invention can be synthesized in an analogous manner, using synthetic modifications known in the art.

### Results of Assays of Test Peptides

#### 5 Example 6 - Binding Assays

In order to demonstrate the binding affinity of somatostatin (SRIF) analogs to the somatostatin receptor, the purified compounds described above were tested in somatostatin binding assays involving measurements of the  
10 in vitro inhibition of the binding of [ $^{125}\text{I}$ -Tyr $^{11}$ ]SRIF-14 to rat AR42J pancreas membranes. As indicated in Table I, purified somatostatin analogs of this invention demonstrated high binding affinities to these receptors. Additionally, the molecular weight, determined by mass  
15 spectrometry and estimated from the molecular structure, is listed in the table for each somatostatin derivative.

Similarly, the purified bombesin analog described above was tested in a bombesin binding assay. The binding assay consisted of measurements of the in vitro  
20 inhibition of the binding of [ $^{125}\text{I}$ -Tyr $^{11}$ ] bombesin to rat AR42J pancreas membranes; from the assay, the binding affinity of the bombesin analog to the GRP receptor was determined to be about 21 nM.

#### 25 Example 7 - Growth Hormone (GH) Inhibition Assay

Groups of five male Sprague Dawley rats (each having a weight between 250-300 g) were injected s.c. with a somatostatin derivative or saline. Thirty minutes prior to the selected post-drug time periods shown in  
30 table II (2 hours, 4 hours, 6 hours, 8 hours), rats were anesthetized with Nembutal i.p. (50 mg/kg). Fifteen minutes following anesthesia, an aliquot of blood was withdrawn by cardiac puncture over heparin to measure basal GH. Additionally, a s.c. injection of D-Ala $^2$ -GRF  
35 (10  $\mu\text{g/kg}$ ) was given. Fifteen minutes later, blood was



- 31 -

withdrawn to quantitate the stimulated GH, which was measured in plasma using a radioimmunoassay supplied by NIADDKD. The percentage of GH inhibition was calculated from differences obtained between basal and stimulated GH values.

Table II shows the effect of various purified somatostatin analogs as a function of time. The efficacy of D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH<sub>2</sub> (BIM-23060) in inhibiting growth hormone in rats is compared with other somatostatin derivatives (BIM-23167, BIM-23179, and BIM-23181) of the invention. All derivatives demonstrate a surprising prolonged duration of action which decreases in a time-dependent fashion.

Additional experiments were conducted on D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH<sub>2</sub>, a somatostatin analog, and BIM-23190, BIM-23195 and BIM-23197, to determine the ED<sub>50</sub> (i.e., the concentration of each compound required to inhibit fifty percent of growth hormone release after a specified time) of the respective compound. Experiments were conducted at a dose range of between 25 µg/kg and 0.25 µg/kg. Table III shows the surprising improvement of the somatostatin derivatives over the unmodified peptide at the various time intervals, indicating the time-dependent inhibition of stimulated GH release by the compounds of the invention.

#### Example 8 - Antiproliferative Assay

The purified somatostatin analogs described above were also tested for activity against rapidly proliferating cells. Table IV describes the effect of these peptides on the growth of AR42J rat pancreas tumor cells. Unlike natural somatostatin, the derivatives of the invention demonstrate substantial anti-proliferative activity. Referring now to Fig. 1, both BIM-23014C (a somatostatin analog) and BIM-23118 (a derivative of BIM-

- 32 -

23014) inhibit the growth of AR42J rat pancreas tumor cells in a concentration-dependent fashion, with BIM-23118 being the more effective of the two compounds. Both compounds inhibit tumor cell growth to a greater extent than unmodified somatostatin analogs at equivalent concentrations.

#### Example 9 - Thymidine Uptake Assay

In this assay, stock cultures of Swiss 3T3 cells are grown in Dulbecco's Modified Eagles Medium (DMEM) and supplemented with 10% fetal calf serum in a humidified atmosphere of 10% CO<sub>2</sub> and 90% air at 37°C. Cells were then seeded into 24-well cluster trays and used four days after the last change of medium. In order to arrest cells in the G1/G0 phase of the cell cycle, the a serum-free DMEM was used 24 hours prior to the thymidine uptake assay; cells were then washed twice with 1 ml aliquots of DMEM (-serum, 0.5 µM) and [methyl-<sup>3</sup>H] thymidine (20Ci/mMole, New England Nuclear). Bombesin derivatives were initially tested at 0.001, 0.01, 0.1, 1, 10, 100, 100 nM. After 28 hours at 37°C, [methyl-<sup>3</sup>H] thymidine incorporation into acid-insoluble pools was assayed as follows. Cells were first washed twice with ice-cold 0.9% NaCl (1 ml aliquots); acid-soluble radioactivity was then removed by 30-minute incubation at 40°C with 5% trichloroacetic acid (TCA). The cultures were then washed once (1 ml) with 95% ethanol and solubilized by a 30-minute incubation with 1 ml of 0.1N NaOH. The solubilized material was transferred to vials containing 10 ml ScintA (Packard), and the radioactivity determined by liquid scintillation spectrometry. This assay demonstrates the ability of the bombesin derivatives to stimulate thymidine uptake into the cells. The EC<sub>50</sub> was

- 33 -

calculated to be 0.48 nm, thus demonstrating that the bombesin derivatives of the invention are potent simulators of thymidine uptake.

#### Methods of Use

- 5           The peptide derivatives of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained-release formulation using a biodegradable, biocompatible polymer,
- 10 or by on-site delivery (e.g., in the case of an anti-cancer bombesin or somatostatin derivatives, to the lungs) using micelles, gels and liposomes. Dosages are generally the same as those currently used for therapeutic peptides in humans.
- 15           Additionally, the peptide derivatives of the invention are suitable for the improved treatment of diseases which are susceptible to treatment by the corresponding unmodified peptide. In particular, the somatostatin derivatives described above are suitable for
- 20 the treatment of cancer, acromegaly, pancreatitis, trauma induced proliferation, diabetes, diabetic retinopathy, restenosis following angioplasty, AIDS, neurogenic inflammation, arteritis, and gastrointestinal problems including diarrhea.

- 34 -

**TABLE I-IN VITRO BINDING AFFINITIES AND MOLECULAR WEIGHTS  
OF SOMATOSTATIN PEPTIDE DERIVATIVES**

		MW TEST	MW CALC	IC <sub>50</sub> nM
	SRIF - 14	-	-	0.17
	SRIF - 28	-	-	0.23
5	BIM - 23107	1340.4	1340.40	0.30
	BIM - 23118	1313.5	1313.52	0.30
	BIM - 23135	1426.2	1426.64	2.52
	BIM - 23158	1299.6	1299.54	0.33
	BIM - 23167	1347.6	1347.55	0.09
10	BIM - 23173	1235.5	1235.46	0.11
	BIM - 23179	1305.9	1305.55	0.12
	BIM - 23181	1435.0	1434.62	0.25
	BIM - 23182	1193.8	1193.42	0.12
	BIM - 23183	1323.0	1322.49	0.22
15	BIM - 23190	1202.8	1202.47	0.20
	BIM - 23191	1314.9	1314.61	0.08
	BIM - 23195	1150.8	1150.39	0.08
	BIM - 23196	1243.7	1243.50	0.09
	BIM - 23197	1252.7	1252.55	0.29
20	BIM - 23201	1262.8	1262.53	0.14
	BIM - 23202	1247.0	1246.53	0.18

SUBSTITUTE SHEET (RULE 26)

- 35 -

TABLE II-INHIBITION OF STIMULATED GROWTH HORMONE RELEASE  
IN RATS BY SOMATOSTATIN PEPTIDE DERIVATIVES

INHIBITION (PERCENTILE CONTROL) 25  $\mu$ G/KG

	2 Hours	4 Hours	6 Hours	8 Hours
BIM-23060	86.39	64.96	47.62	38.15
BIM-23167	92.67	79.54	59.72	50.14
BIM-23179	92.79	63.85	67.78	68.26
BIM-23181	99.24	77.07	60.56	56.12

TABLE III-INHIBITION OF STIMULATED GROWTH HORMONE RELEASE  
IN RATS BY SOMATOSTATIN PEPTIDE DERIVATIVES ADMINISTERED

S.C.

ED 50 ( $\mu$ g/kg)

	2 Hours	4 Hours	6 Hours	8 Hours
BIM-23023	0.48	1.11	2.26	4.32
BIM-23190	0.68	0.57	0.76	1.04
BIM-23195	1.19	3.13	2.08	3.23
BIM-23197	1.01	0.59	1.14	1.59

- 36 -

TABLE IV-ANTIPROLIFERATIVE ACTIVITY OF  
SOMATOSTATIN PEPTIDE DERIVATIVES

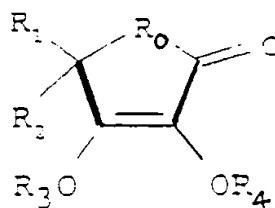
CELL GROWTH (PERCENT OF CONTROL) <sup>1</sup>		
5	SRIF - 14	91.3
	SRIF - 28	98.0
	BIM-23014C	74.1
	BIM-23107	67.5
	BIM-23109	72.1
10	BIM-23118	61.0
	BIM-23135	62.9
	BIM-23167	60.2
	BIM-23173	67.9
	BIM-23181	69.1
15	BIM-23182	68.7
	BIM-23183	69.1
	BIM-23195	69.2
	BIM-23197	66.4

<sup>1</sup> Concentration 100 nM, AR42J Rat Pancreas Tumor Cells.  
after 8 days.

20 What is claimed is:

- 37 -

1. A peptide derivative comprising:  
 a biologically active peptide moiety, and  
 at least one substituent attached to said peptide  
 moiety, wherein said substituent is selected from the  
 group consisting of Compounds I, II and III, wherein  
 5 Compound I is:

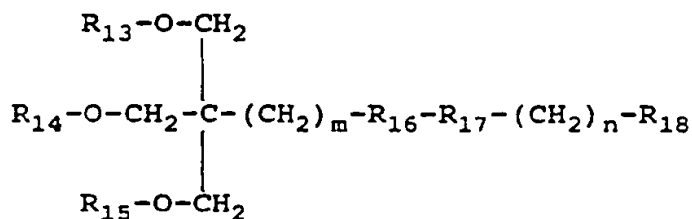


wherein :

- $R_0$  is O, S, or  $NR_5$ , wherein  $R_5$  is H or  $(C_1-C_6)$  alkyl;  
 10 each  $R_1$  and  $R_2$ , independently, is H,  $(CH_2)_mOR_6$ , or  $CH(OR_7)CH_2OR_8$ , wherein  $R_6$  is H or  $(C_2-C_7)$  acyl, and each  $R_7$  and  $R_8$ , independently, is H,  $(C_2-C_7)$  acyl, or  $C(R_9)(R_{10})$ , wherein each  $R_9$  and  $R_{10}$ , independently, is H or  $(C_1-C_6)$  alkyl;  
 15 or each  $R_1$  and  $R_2$  is  $=CHCH_2OR_{11}$ , wherein in  $R_1$  and  $R_2$  independently,  $R_{11}$  is H or  $(C_2-C_7)$  acyl, and  $m$  is an integer between 1 and 5, inclusive; and  
 20 one of  $R_3$  or  $R_4$  is  $(CH_2)_nR_{12}$  or  $(CH_2)_nCH(OH)R_{12}$ , wherein  $R_{12}$  is CO,  $CH_2$ , or  $SO_2$ , and  $n$  is an integer between 1 and 5, inclusive;  
 and the remaining  $R_3$  or  $R_4$  is H,  $(C_1-C_6)$ hydroxyalkyl, or  $(C_2-C_7)$  acyl;  
 and

- 38 -

Compound II is:



5 wherein:

each  $R_{13}$ ,  $R_{14}$  and  $R_{15}$ , independently, is H or ( $C_2$ - $C_{24}$ ) acyl;

$R_{16}$  is NH or absent;

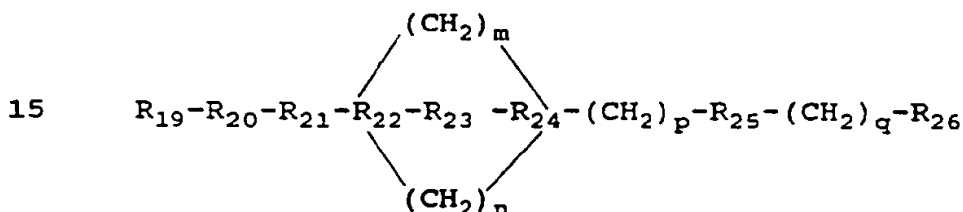
$R_{17}$  is CO, O, or absent;

10  $R_{18}$  is CO,  $CH_2$ ,  $SO_2$ , or absent;

$m$  is an integer between 1 and 5, inclusive;

$n$  is an integer between 0 and 5, inclusive; and

Compound III is:



wherein:

$R_{19}$  is H,  $NH_2$ , an aromatic functional group, OH, ( $C_1$ - $C_6$ )hydroxyalkyl,  $H(R_{27})(R_{28})$ ,

20  $SO_3H$ , or absent; wherein each  $R_{27}$  and  $R_{28}$ , independently, is H or ( $C_1$ - $C_6$ ) alkyl;

$R_{20}$  is O or absent;

$R_{21}$  is ( $C_1$ - $C_6$ )alkyl or absent;

$R_{22}$  is N, O, C, or CH;

25  $-R_{23}-$  is ( $C_1$ - $C_6$ )alkyl or absent;

$R_{24}$  is N, CH, or C;

$R_{25}$  is NH, O, or absent;

$R_{26}$  is  $SO_2$ , CO,  $CH_2$ , or absent;



- 39 -

m is an integer between 0 and 5, inclusive;  
n is an integer between 0 and 5, inclusive;  
p is an integer between 0 and 5, inclusive; and  
q is an integer between 0 and 5 inclusive;

5 wherein said peptide moiety is attached to each of said substituents by a CO-N, CH<sub>2</sub>-N, or SO<sub>2</sub>-N bond between said substituent and a nitrogen atom of the N-terminus or a side chain of said peptide moiety.

2. The peptide derivative of claim 1, wherein  
10 said substituent is Compound I.

3. The peptide derivative of claim 2, wherein R<sub>12</sub> is CH<sub>2</sub> or SO<sub>2</sub>.

4. The peptide derivative of claim 1, wherein said substituent is Compound II.

15 5. The peptide derivative of claim 4, wherein R<sub>18</sub> is CH<sub>2</sub> or SO<sub>2</sub>.

6. The peptide derivative of claim 5, wherein R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> are H, and R<sub>17</sub> is absent.

7. The peptide derivative of claim 6, wherein  
20 said substituent is (HOCH<sub>2</sub>)<sub>3</sub>C-NH-(CH)<sub>2</sub>-SO<sub>2</sub> or (HOCH<sub>2</sub>)<sub>3</sub>C-CH<sub>2</sub>.

8. The peptide derivative of claim 1, wherein said substituent is Compound III.

9. The peptide derivative of claim 8, wherein  
25 -R<sub>23</sub>- is (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sub>22</sub> is N, C, or CH; and R<sub>24</sub> is C.

- 40 -

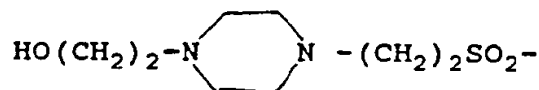
10. The peptide derivative of claim 8, wherein  $R_{22}$  is O;  $R_{19}$ ,  $R_{20}$ ,  $R_{21}$ , and  $-R_{23}-$  are absent; and the sum of m and n is 3, 4, or 5.

11. The peptide derivative of claim 8, wherein  $-R_{23}-$  is absent.

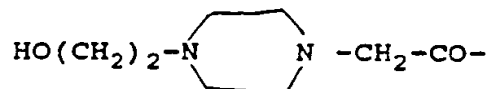
12. The peptide derivative of claim 11, wherein at least one of  $R_{22}$  and  $R_{24}$  is N.

13. The peptide derivative of claim 12, wherein both  $R_{22}$  and  $R_{24}$  are N.

10 14. The peptide derivative of claim 13, wherein said substituent is one of:



and



15 15. The peptide derivative of claim 1, wherein said peptide moiety is selected from the group consisting of: somatostatin, bombesin, calcitonin, calcitonin gene related peptide (CGRP), amylin, parathyroid hormone (PTH), gastrin releasing peptide (GRP), melanocyte  
20 stimulating hormone (MSH), adrenocorticotrophic hormone (ACTH), parathyroid related peptide (PTHrP), luteinizing hormone-releasing hormone (LHRH), growth hormone releasing factor (GHRF), growth hormone releasing peptide (GHRP), cholecystokinin (CCK), glucagon, Bradykinin,  
25 glucagon-like peptide (GLP), gastrin, enkephalin, neuromedins, endothelin, substance P, neuropeptide Y (NPY), peptide YY (PYY), vasoactive intestinal peptide (VIP), guanylin, pituitary adenylate cyclase activating

- 41 -

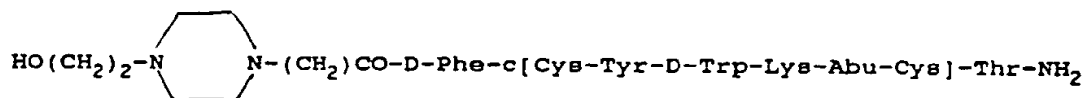
polypeptide (PACAP), beta-cell tropin, adrenomedulin, and derivatives, fragments, and analogs thereof.

16. The peptide derivative of claim 15, wherein said peptide moiety is somatostatin or a derivative, fragment, or analog thereof.

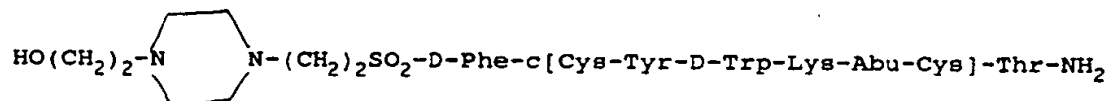
17. The peptide derivative of claim 16, wherein said somatostatin analog is one of: H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH<sub>2</sub>, H-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH<sub>2</sub>, and H-D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH<sub>2</sub>.

18. The peptide derivative of claim 15, wherein said peptide moiety is bombesin or a derivative, fragment or analog thereof.

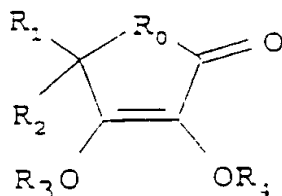
19. The peptide derivative of claim 1, wherein said peptide derivative is one of:



and



20. A dimeric peptide derivative, comprising:  
two biologically active peptide moieties, and  
at least one substituent attached to one of said peptide moieties, wherein said substituent is one of Compounds IV and V, wherein Compound IV is:

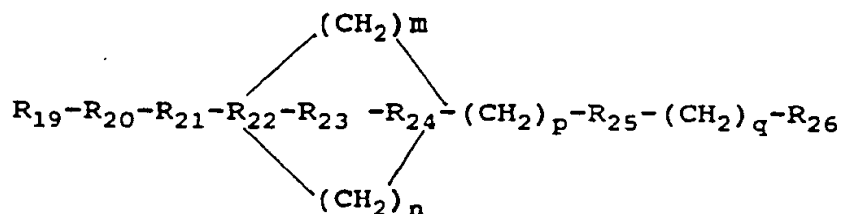


- 42 -

wherein :

- $R_0$  is O, S, or  $NR_5$ , wherein  $R_5$  is H or  $(C_1-C_6)$  alkyl;
- each  $R_1$  and  $R_2$ , independently, is H,  $(CH_2)_mOR_6$ , or  $CH(OR_7)CH_2OR_8$ , wherein  $R_6$  is H or  $(C_2-C_7)$  acyl, and each  $R_7$  and  $R_8$ , independently, is H,  $(C_2-C_7)$  acyl, or  $C(R_9)(R_{10})$ , wherein each  $R_9$  and  $R_{10}$ , independently, is H or  $(C_1-C_6)$  alkyl;
- or each  $R_1$  and  $R_2$  is  $=CHCH_2OR_{11}$ , wherein  $R_{11}$  in  $R_1$  and  $R_2$ , independently, is H or  $(C_2-C_7)$  acyl, and  $m$  is an integer between 1 and 5, inclusive; and each  $R_3$  or  $R_4$ , independently, is  $(CH_2)_nR_{12}$  or  $(CH_2)_nCH(OH)R_{12}$ , wherein  $R_{12}$  is CO,  $CH_2$ , or  $SO_2$ , and  $n$  is an integer between 1 and 5, inclusive; and,

Compound V is:



wherein:

- $R_{19}$  is  $SO_2$ , CO, or  $CH_2$ ;
- $R_{20}$  is O or absent;
- $R_{21}$  is  $(C_1-C_6)$  alkyl or absent;
- $R_{22}$  is N, CH, O, or C;
- $-R_{23}-$  is  $(C_1-C_6)$  alkyl or absent;
- $R_{24}$  is N, CH, or C;
- $R_{25}$  is NH, O, or absent;
- $R_{26}$  is  $SO_2$ , CO,  $CH_2$ , or absent;
- $m$  is an integer between 0 and 5, inclusive;
- $n$  is an integer between 0 and 5, inclusive;

- 43 -

p is an integer between 0 and 5, inclusive;  
q is an integer between 0 and 5, inclusive; and  
wherein at least one of said peptide moieties is  
attached to each of said substituents by a CO-N, CH<sub>2</sub>-N,  
5 or SO<sub>2</sub>-N bond between said substituent and a nitrogen  
atom of either the N-terminus or a side chain of one of  
said peptide moieties.

21. The dimeric peptide derivative of claim 20,  
wherein -R<sub>23</sub>- is (C<sub>1</sub>-C<sub>6</sub>)alkyl; R<sub>22</sub> is N, C or CH; and R<sub>24</sub>  
10 is C.

22. The dimeric peptide derivative of claim 20,  
wherein R<sub>22</sub> is O; R<sub>19</sub>, R<sub>20</sub>, R<sub>21</sub> and -R<sub>23</sub>- are absent; and  
the sum of m and n is 3, 4, or 5.

23. A method of treating a disease of a patient,  
15 comprising the step of administering to said patient a  
therapeutic amount of the peptide derivative of claim 1.

24. The method of claim 23, wherein said peptide  
moiety is somatostatin or an analog thereof.

25. The method of claim 23, wherein said disease  
20 is cancer.

1/1

### Effect of Somatostatin Analogs on the Proliferation of AR42J Rat Pancreatic Tumor Cells

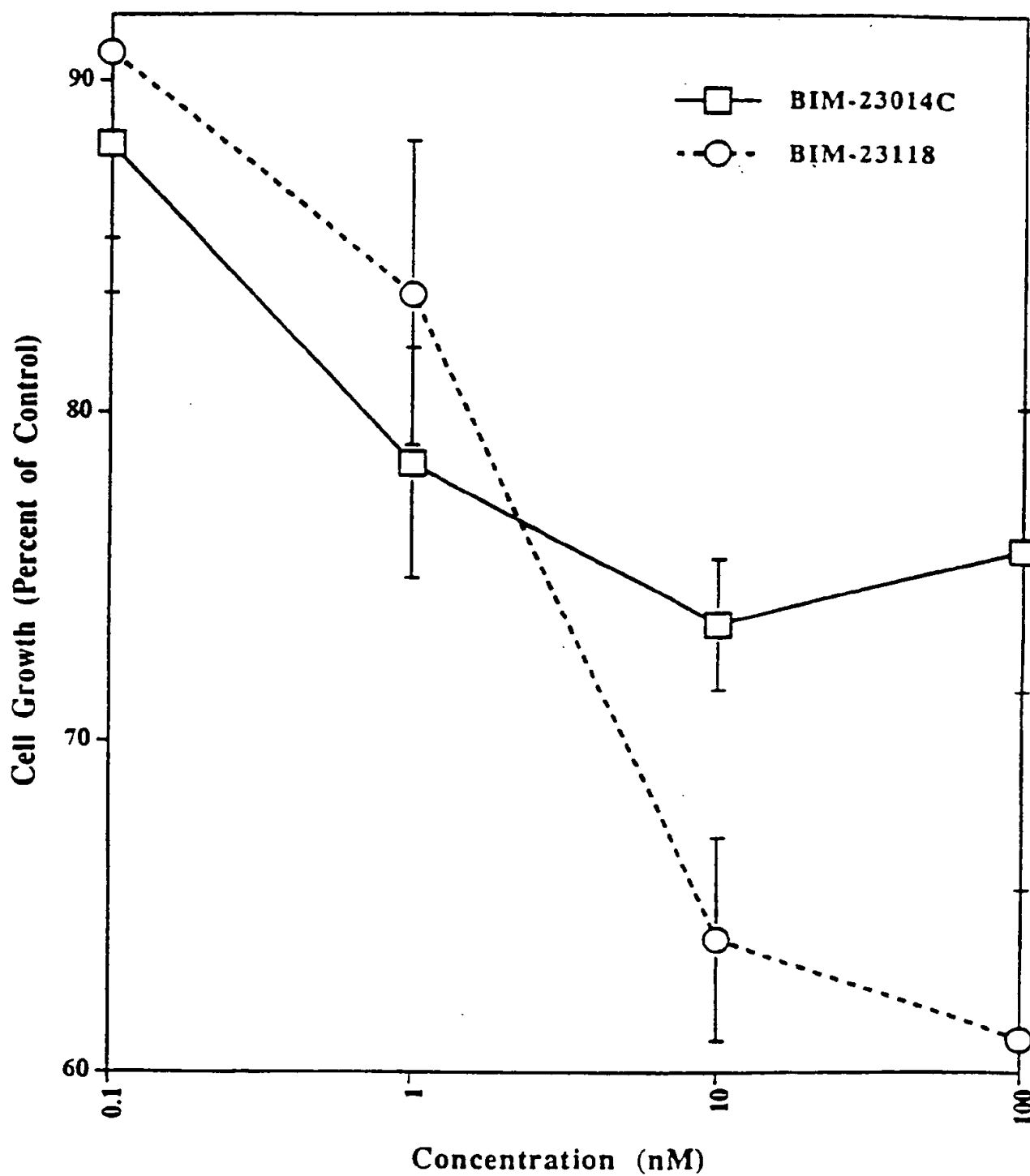


Fig. 1

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/08875

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 13, 14, 15, 16, 21; 530/302, 306, 307, 308, 311, 324, 345, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 88/02756 (SANDOZ AG) 21 April 1988, see entire document.	1-25
X	WO, A, 89/09786 (ALBERT ET AL) 19 October 1989, see entire document.	1-25
X	US, A, 4,837,303 (JUNG) 06 June 1989, see entire document.	1-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

22 SEPTEMBER 1994

Date of mailing of the international search report

17 OCT 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

CAROL A. SALATA

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08875

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (5):

C07K 5/04, 7/10, 7/34, 7/36, 7/44, 7/26, 7/38, 7/12; A61K 37/24, 37/28, 37/40

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

514/12, 13, 14, 15, 16, 21; 530/302, 306, 307, 308, 311, 324, 345, 350